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Research Proposal Submitted to the

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by

Indiana University Foundation
P. O. Box 1847
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For scientific review of research grant NGR 15-003-118, entitled

Isotopic Biogeochemistry

Progress report for 1 June 1981 - 30 April 1985
Plans for 1 May 1985 - 30 November 1988

Principal Investigator: J. M. Hayes
Professor of Biogeochemistry
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Propos FOR PROGRESS REPORT

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2. Brian D. Fry, Postdoctoral Research Associate
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Introduction

This document includes both a progress report and a proposal for future work. An extended introduction provides an overview of the biogeochemical research group at Indiana University -- its funding, productivity, personnel, and facilities -- and contains information that reviewers will need to place the progress report and proposal in context. Appendices bound separately (see Table of Contents) provide additional documentation. Citations in brackets [] refer to the appendices.

The work funded by NGR 15-003-118 was last reviewed in May, 1981. The proposal considered at that time covered the period 1 June 1981 - 31 May 1982. Since then, additional funding has been received for the periods 1 June 1982 - 31 May 1983 and 1 June 1983 - 30 November 1984. We are presently in a period of provisional funding (no triannual review having occurred) covering the period 1 December 1984 - 30 November 1985. The progress report offered here covers the period from the last review until 30 April 1985.

Funding. Funding from all sources is summarized in Appendix F, which also reports proposals presently under consideration and awards for future work. Even though funds from this grant have amounted to just over half the total available to the group, an analysis of publications (below) shows that it represents the heart of our effort. That position derives, of course, from its alignment with our principal scientific interests, but also from the wonderful continuity and stability which it has provided. The principal investigator, his research colleagues, and students are all sincerely grateful for that continuity, without which a program with depth and intensity

cannot be maintained. It is our goal here to justify the renewal of that support.

Productivity. Based on the source of funds utilized for salaries, stipends, nd support of research facilities, our published work should be associated with various projects as follows (numbers and letters refer to entries in the PI's bibliography, appendix E):

Related entirely to this project	55, 57, 60, 63, 68, 69, 70, 74, a, e, g
50:50, this project and Precambrian Paleobiology Research Group	59, 61, 62
Related entirely to PPRG	64, 65, 66
50:50, this project and NSF-funded work of H. Geat	67, 71, f
50:50, this project and EPA-funded work on isotopic analysis of oxygen in organic materials	b, c
50:50, this project and other sources	(Vogler et al., 1981)
25:75, this project and other NSF-funded work with Geat	d
25:75, this project and other sources	(Fry and Sherr, 1984)
Related to this project, but funded entirely by other sources	56, 58
DOE-funded work on mobility of organic materials in ground water	72, 73

In addition, we have provided isotopic analyses utilized in publications by others working in related fields. These contributions are cited at appropriate points in the progress report.

Relationships to Other Projects

PPRG. Aspects of this work are related to that of the Precambrian Paleobiology Research Group, funded through UCLA and the University of Michigan. During the interval covered by this progress report, PPRG funds have been utilized only for field work. New proposals for "PPRG - Proterozoic"

include requests for direct support [F] of work at Indiana. Specifically, funds for isotopic geochemical analyses and assembly of related databases are sought from NSF, and support for studies of isotope effects associated with microbial communities of paleobiological interest is sought from NASA. The latter work will be carried out in partnership with Dr. Howard Gest, Distinguished Professor of Microbiology at IU. The geochemical work will be supervised in our laboratories by Dr. Harald Strauss, a new postdoctoral fellow planning to join our group in 1986. In order to provide more complete background information, Dr. Strauss's curriculum vita is appended here [E], although he will not be supported by funds from this proposal.

Microbial biogeochemistry of sulfur. Extensive collaboration has developed between our laboratory and that of Professor Gest. The catalyst has been Brian Fry [V], who joined both groups as a post-doctoral research associate in September, 1982. Half of Dr. Fry's support derived from this project until 15 July 1984, when additional funding was obtained from NSF [F]. The new NSF project has the measurement of sulfur isotope effects (primarily those associated with the oxidative side of the sulfur cycle) in microbial systems as its goal. Carbon isotopic fractionations occurring in the same systems are also being studied.

Mobility of organic compounds in ground water. The Department of Energy (through its contractors and organizational predecessors, the AEC and ERDA) supported work in our laboratories on the abundance and transport of halocarbons in near-surface geochemical systems. This project ended during the first year of the report period [F] and has had no impact on laboratory operations since then, but publications from it (72, 73) are still working

through the system.

Oxygen-18 as a tracer in studies of inhalation toxicology. Because of our expertise in isotopic analyses, we were asked by the Environmental Protection Agency to take up a project aimed at determining the fate of ozone-derived oxygen in mammalian respiratory systems. This project seized the interest of Jeffrey Santrock, a graduate student in analytical chemistry who has just completed a superb doctoral thesis on this subject, demonstrating, for the first time, the use of ^{18}O as a low-level tracer detectable even in total-tissue oxygen (including water). As a result of this success, our funding [F] survived even the EPA cuts of the first Reagan term.

We benefited in several ways. The income contributed significantly to meeting the costs of research group operations. An expensive elemental analyzer of broad use in our work became available through this project. Portions of salaries (summer-PI, technicians) were paid by the EPA. Significant analytical developments [B-2, B-3] relevant to our biogeochemical work have resulted.

But the work also constituted a significant distraction. An elaborate and difficult method of analysis for ^{18}O in organic materials (Santrock and Hayes, manuscript in preparation) that meets the requirements (high speed, low cost per analysis, adequate precision when applied to materials with high oxygen content) of physiological tracer work has proved inadequate for biogeochemical use. The very large numbers of samples analyzed in connection with specific physiological studies (Santrock and EPA coworkers, manuscripts in preparation) have consumed much of the additional laboratory support noted above.

Isotope-ratio monitoring GCMS. Matthews and Hayes (45) demonstrated that variations in the natural abundance of ^{13}C among compounds could be measured by a specialized gas chromatograph-mass spectrometer system in which the chromatographic effluent was passed through a combustion furnace in order to produce CO_2 for continuous isotopic analysis. Earlier investigations by Welte (1969), DesMarais et al. (53), and Vogler et al. [A-19] demonstrated the importance of this capability for organic geochemical work. Unfortunately, due to the poor sensitivity of the isotope-ratio mass spectrometer utilized, precision was marginal, large samples were required, and high-resolution chromatographic techniques could not be employed. Solution of these problems requires extensive mass spectrometric and other analytical developments. A project now initiated [F] incorporates funds for purchase of a specialized mass spectrometer and studies of its applicability (Chevron Oil Field Research Corporation) and donations of engineering expertise and funds for refinement of the mass spectrometer system (Finnigan MAT, a developer and manufacturer of mass spectrometers). We expect this project to interact very constructively with our biogeochemical work.

Personnel and Facilities

Permanent staff. On 15 August 1984, the academic appointment of the principal investigator was changed from Professor of Chemistry, with a courtesy appointment in geology (i. e., no teaching responsibilities or formal departmental role, but access to graduate students), to Professor of Biogeochemistry, with regular membership in both departments. This change is consistent with the development of our research and, most of all, with the background and interests of the graduate students and postdoctoral research

associates entering the group. It also offers the stimulation of half-time teaching in the department of geology, rather than still more freshman chemistry.

Stephen Studley joined the group as a dishwasher in 1971 and has been with us ever since. Over the years, he has developed into a superb mass spectroscopist, isotopic analyst, and computer programmer. His judgment, intelligence, independence, initiative, and maturity mark him as a professional colleague, not a technical employee. The university recognized these accomplishments and traits when, in the spring of 1984, Mr. Studley was named as the outstanding technical and clerical staff member in the university (the seventh largest in the country). At this time, we were able to get his position converted to a "hard-money" line, now guaranteeing that if Hayes is wiped out by a truck somewhere between the chemistry and geology buildings, Studley will still have a job. The security provided to Mr. Studley, whose oldest child is nearing college age, is long overdue. In recognition of the value of services that he provides specifically to our group, we want to keep paying half of his salary, but this has been built into the isotope-ratio-monitoring GCMS project described above. The savings to this research grant are considerable, and the loss is near zero.

Graduate students and research associates. The present composition of the group is described in Appendix D. One new graduate student, Ms. Susan Janes, who has just completed an S. B. in earth sciences and chemistry at M. I. T., will be joining the group in the fall. Dr. Brian Fry (noted above in connection with our work on the microbial biogeochemistry of the sulfur isotopes) will move in the fall of 1985 to take up a position of richly deserved independence as a member of the research staff of the Marine Bio-

logical Laboratory, Woods Hole. We have already noted that Dr. Harald Strauss, from the research group of Prof. Jochen Hoefs, University of Gottingen, will join the group in January, 1986. A second new post-doctoral research associate will be Dr. Brian Popp, from the research group of Prof. Tom Anderson, University of Illinois. Dr. Popp will join the group in August, 1985, in order to begin work described in the research-plans section of this proposal. Similarly, Mr. Henrik Fossing, a graduate student from the research group of Dr. Bo Barker Jorgensen at the University of Aarhus, Denmark, will join the group for nine months beginning in July of 1985 in order to work on sulfur-isotope exchange phenomena described in the research-plans section of this proposal.

Facilities and research environment. The research group will move to newly remodeled laboratories in the geology building sometime in the winter of 1985-86. Approximately 1,750 ft^2 of space specifically adapted to our research activities should provide ideal facilities for our geochemical work, far superior to laboratories presently in use. A separate area of 900 ft^2 will accommodate a new departmental facility for isotopic mass spectrometry, pooling instruments presently housed in our laboratories and in those of Prof. Edward Ripley. Mr. Studley will be in charge of that facility. Other aspects of the Bloomington research environment are described in Appendix D.

Carbon-Isotopic Record

PPRG-related work. At the time of the previous review of this work, the manuscript [since published, A-7] summarizing the principal isotopic- and organic-geochemical results of the 1979-1980 investigations had just been completed. In the fall of 1981, intensive work on the isotopic-geochemical data [A-7, A-8] led to the development of the hypothesis [A-9] that the significant excursion in the record of carbon-isotopic abundances in organic meterial about 2.8 Ga ago recorded widespread utilization of biogenic methane as a principal carbon source and, therefore, the first appearance of the O_2. It is conceivable, but not likely [A-9], that this very extensive utilization of CH_4 as a carbon source is linked to activities of sulfate-reducing bacteria.

Further considerations of the carbon-isotopic record have only been presented orally. Briefly, it is likely that reductants serving as redox partners for carbon would have been used in order of reduction potential: H_2, then H_2S, S, Fe^{2+}, and finally H_2O. If so, utilization of H_2O (yielding O_2) ought to have begun only after supplies of all better reductants had been exhausted, probably significantly later than 2.8 Ga ago. Walker (1980) has noted, however, that reduced sulfur would have been inaccessible in a system in which most iron was in the ferrous state and soluble enough to enter solution and scavenge any sulfide (the crustal abundance of Fe is about 100X that of S). In that case, rates of turnover in the carbon cycle might have been limited by the scarcity of mobile redox partners. Driven by this geochemical imperative, use of H_2O as an electron donor may have become quantitatively more important than use of unoxidized S. When? Immediately

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after the evolution of systems able to produce and deal with O_2. The onset of O_2 production would have been followed by a long period during which O_2, a superbly mobile oxidant, slowly collected electrons from unoxidized constituents of the crust. During this interval, O_2 would have been an important trace constituent of the atmosphere, turning over rapidly, but rarely accumulating. This interpretation would be consistent with the isotopic record [A-7, A-8]. Clarification is likely to come from study of some line of evidence other than the carbon-isotopic record. The nitrogen-isotopic record, budgets of redox partners, and sedimentary petrology would all seem to be good bets.

The first PPRG field trip after the 1979-1980 year returned, in June, 1982, to the Pilbara region of northern Western Australia. Several hundred new samples were obtained, brought back to Bloomington, and analyzed in the same way that samples had been analyzed at UCLA. Results obtained confirmed those obtained in 1979-1980. In addition: (i) the "Fortescue Group light-carbon feature" ($\delta^{13}C_{PDB}$ values near -50‰) was extended to all exposures from which materials of that age could be collected; (ii) a few samples even more strongly depleted in ^{13}C were found (δ to -64‰), these tended to be near the bottom of the Fortescue Group; (iii) a significant collection of Archean carbonates was assembled, and it was found that isotopic compositions of all were near those of more recent marine carbonates ($\delta^{13}C \sim 0$‰). The last finding, in particular, may be worthy of separate publication. These materials await further investigation by Dr. Strauss.

The "PPRG-style" analysis of samples from the 1982 field trip was a

significant experience for our laboratories. We developed new techniques (still unpublished because we keep tinkering with the procedure, see section on research plans) for measurement of abundance and isotopic composition of total organic carbon in sediments. Dealing with the flood of data, we turned to the use of (then) "dBase II," an item of microcomputer software that quickly became a mainstay. Using it, and its much-improved successor, "dBase III," we have developed a sample-tracking and data-logging system of great value to us. The "structure" of that database, called "PCSAMP," is included in the appendix [G-1].

Late Proterozoic section in Svalbard and East Greenland. Working in collaboration with Prof. Andrew Knoll, Harvard University, and Prof. Keene Swett, University of Iowa, we have been exploring the isotopic record of organic and inorganic carbon in sediments from the locations noted for the time interval 900 - 600 Ma ago. Hundreds of analyses have been carried out in our laboratories by Jay Kaufman. We have noted a significant enrichment of ^{13}C in both carbonate and organic carbon in this interval, with episodic returns to "normal marine" isotopic compositions. This pattern appears to fit well with global trends described by Holser (1984) for the Phanerozoic, and can be compared to that noted at the close of the Permian (Margaritz et al., 1983). A manuscript is in preparation. To avoid extending this section of our report, we refer interested readers to a "capsule description" of that manuscript prepared by Knoll and Hayes and included with this proposal [G-2]. Further work planned for the future is described in a later section of this proposal.

The Early Proterozoic section near Danielskuil, North Cape Province, South Africa. A second interval in which study of isotopic compositions of

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carbonates and coexisting organic material appeared to be of interest was located in the Gamohaan Formation of the Campbellrand Subgroup, Ghaap Group, Transvaal Supergroup, ~ 2.3 Ga in age. Materials exposed in this section were rich in structural detail and appeared to be much better preserved than many much younger carbonate sequences. With the assistance of Prof. N. Beukes, Rand Afrikaans University, materials for an isotopic reconnaissance of this section were collected during the 1984 PPRG field trip. The petrography and isotopic compositions of these materials have been studied in our laboratories by J. A. Baier. An abbreviated report is attached [G-3]. Unfortunately, microscopic reality has turned out to be quite different from macroscopic promise. Both carbonates and organic materials appear significantly altered and are isotopically homogeneous. At present, we plan no further, detailed studies of this section. and other

Assistance to others. Approximately 60 different samples from the Archean sequence in the Barberton Mountain Land of South Africa have been analyzed for Ms. Maud Walsh, a graduate student working with Prof. Don Lowe, Louisiana State University (Walsh and Lowe, 1985). Samples of Middle Proterozoic stromatolites from the Gaoyuzhuang Formation, near Jixian, Northern China, have been analyzed for Prof. J. W. Schopf, UCLA (Schopf et al., 1984).

Isotopic Studies of Banded Iron Formations

The work of Baur, Hayes, Studley, and Walter, begun during the 1979-80 PPRG year, was completed during the period covered by this progress report. Our report has just appeared in Economic Geology [A-18]. In brief, we offer the following interpretation (for details and relevant citations, please see

appendix): carbonates in banded iron-formations are depleted in ^{13}C because they incorporate carbon derived from organic material; that "light" carbonate was produced below the sediment-water interface by heterotrophic organisms possibly using Fe^{3+} as an electron acceptor; it is, therefore, possible, even though the average oxidation level of iron in iron-formations is ~2.5, that iron was initially precipitated from solution by oxidative processes. Based on his study of our preprint, Walker (1984) offered a closely related interpretation. It is, apparently, not a bad idea.

More recently, working with the support of this grant, Kaufman, Klein, and Hayes have shown that millimeter-scale variations of carbon and oxygen isotopes in the Dales Gorge Member of the Brockman Iron-Formation (not studied by Baur et al.) are very closely correlated. This linkage is difficult to attribute to any mechanism other than evaporation. A report and discussion [C-3] of this observation have just been prepared for submission to Nature.

We are very encouraged by the progress of our work on banded iron-formations (much credit must go to Professor Klein). The probable importance of these sediments as indicators of a major transition in global geochemistry has been recognized by many workers before us. In spite of extensive study, however, details of the origin of BIFs and the nature of their linkage to undoubted major environmental changes have remained enigmatic. We do not think that we are more able than our predecessors, but we do think that isotopic analyses carried out with high spatial resolution are revealing important new information, and that the combination of an experienced petrologist with a free-thinking (?) biogeochemist is leading to exploration of some new possibilities.

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Isotope Effects in Microbial Systems

Carbon isotopic fractionation in lipid biosynthesis. David Monson completed his doctoral work in the summer of 1981. Manuscripts resulting from that work were prepared, and papers have appeared, during the period covered by this progress report.

Monson was able to define conditions [A-1] for quantitative conversion to carbon dioxide of olefinic carbon positions in unsaturated fatty acids. As a result, intramolecular isotopic analyses within hydrocarbon chains became possible, and Monson showed that alkyl chains produced by the anaerobic pathway of fatty-acid biosynthesis contained two subsets of carbon positions. One, occupied by carbon from the methyl position of acetyl-coenzyme A, was enriched in ^{13}C relative to the other, occupied by carbon from the carboxyl position in acetyl-CoA. It is logical to suggest [A-1] that this results from a carbon kinetic isotope effect at C-2 in the decarboxylation of pyruvate by pyruvate dehydrogenase. An effect at this point had already been postulated by DeNiro and Epstein (1977). Monson showed that, *in vivo*, this effect must amount to 2.32% and that quantitative modeling based on that value and on a similarly-determined value for the isotope effect at the chain-elongation *vs.* transesterification branch point could account for all details of the distribution of carbon isotopes within fatty acids synthesized by *Escherechia coli* grown on glucose [A-1]. It is significant that chemically equivalent positions within the lipid carbon skeletons had very different carbon-isotopic compositions, and that the quantitative model noted above was based on kinetic considerations. Both of these observations are inconsistent with the equilibrium-thermodynamic theory of isotopic par-

titioning developed by Galimov (1985).

Later work by Monson [A-3] took up the problem of carbon-isotopic fractionation during aerobic biosynthesis of fatty acids by Saccharomyces cerevisiae, a eukaryotic organism. In this case, it was apparent that the unsaturated carbon positions were strongly depleted in ^{13}C and not isotopically representative of the remainder of the hydrocarbon chain. Construction of a detailed kinetic model showed that the carbon-isotopic compositions tightly constrained carbon flows within the complicated pathways of eukaryotic lipid biosynthesis and, notably, required a very large turnover (synthesis and degradation at least to the level of C_2 units) of C_{18} carbon skeletons. These results have significance for lipid biochemistry, apparently offering a new means of recognizing the extent of peroxisomal activity, and for biogeochemistry, providing an instructive example of reconstruction of carbon flows within a complex reaction network and offering a means of distinguishing between lipids with identical structures but different origins (eukaryotic vs. prokaryotic).

In connection with his work on isotopic compositions of individual organic compounds in sediments (the objectives of this work are introduced in the research-plans section of this proposal), Ray Takigiku has studied isotopic compositions of biosynthetic products in both photo- and chemoautotrophs. Two groups of compounds have been of principal interest: (i) possible precursors of sedimentary porphyrins, and (ii) archaebacterial lipids.

Isotopic compositions of chlorophyllides (= chlorophyll - esterifying polyisoprenoid alcohol) produced by higher-plant chloroplasts (beech tree) and by anaerobic photosynthetic bacteria (Rhodopseudomonas capsulata and

Chromatium vinosum grown at 32°C in Prof. H. Gest's laboratories) were within 1‰ of cellular averages in each case. The phytol was further depleted in ^{13}C (4.0 to 4.7‰). The tetrapyrrole pigment produced by methanogens, F_{430} (examined in Methanobacterium thermoautotrophicum, kindly grown for us by Prof. R. S. Wolfe, University of Illinois, T = 65°C -- the difficult isolation of F_{430} was carried out by Trish Hartzell, a student in the Wolfe group) is enriched in ^{13}C relative to biomass by 7.8‰ (in these experiments, methanogenic biomass was found to be depleted ^{13}C relative to the CO_2 substrate by 19.4‰). Lipids produced by M. thermoautotrophicum were depleted in ^{13}C relative to the cellular average by 7.7‰ and were, thus, 15.5‰ lighter than F_{430} synthesized at the same time.

CO_2, CH_4, and CH_3CO_2H in anaerobic microbial communities. Isotope effects associated with the production of methane by M. thermoautotrophicum were redetermined as a byproduct of the investigation described above. Working in very small, closed systems more than 10 years ago, Games, Hayes, and Gunsalus (44) had reported $\alpha(CO_2/CH_4) = 1.025 \pm 0.002$ at 65°C. Working in a large, open system (which they mistakenly termed "closed," depending on reaction conditions), Fuchs et al. (1979) reported a value of 1.024 for the same factor. The new value (this work: Takigiku, Hartzell, Wolfe, and Hayes), also obtained in a large, open system, is also 1.024 and fits well on an open-system fractionation plot which we constructed from the data of Fuchs et al. Clearly, for M. thermoautotrophicum at 65°C, $\alpha(CO_2/CH_4) = 1.024$.

Working with Dr. J. B. Risatti, a geomicrobiologist at the Illinois State Geological Survey, we have further explored carbon isotopic relation-

ships between CO_2, acetate, and methane. Methane is formed in anaerobic microbial communities by the flow of carbon through three different pathways: (i) direct reduction of CO_2 to CH_4; (ii) dissimilation of acetate to form CH_4 and CO_2; and (iii) catabolism of C_1 compounds such as CH_3OH, CH_3SH, and methyl amines. Although it is known from studies using ^{14}C-labeled acetate that the second of these pathways is usually the most important in freshwater sediments, the isotopic characteristics of that pathway have remained unstudied largely because the related organisms have been poorly known and difficult to grow in the laboratory. A second serious problem associated with the acetate-dissimilation pathway has concerned the source of the acetate. The amounts required are very large, and, in order to be consistent with observed isotopic compositions of methane, the isotopic composition of the acetate must be very different from that of most of the organic material in sediments.

We have now measured the carbon isotope effects associated with two previously unexamined steps in bacterial methanogenesis: the dissimilation of acetate, and the production of acetate by "acetogenic bacteria," organisms which utilize H_2 to reduce CO_2 to acetate rather than to CH_4. In each case, we have measured seperately the isotope effects at the methyl and carboxyl carbons of acetate. We have found that both the synthesis and the dissimilation of acetate are accompanied by substantial isotope effects. Acetic acid produced by reduction of CO_2 by Acetobacterium woodii is equal in isotopic composition at both the methyl- and carboxyl-carbon positions, but the isotope effect associated with uptake of CO_2 is large, ϵ = -37.1 ± 1.5‰. The isotope effect associated with the splitting of acetate by Methanosarcina barkeri is asymmetrical, amounting to 29.6 ± 1.0‰ at the methyl group and 0 at the carboxyl group. The latter observation is quite

surprising and seems to require that the rate-determining step in the dissimilation of acetate involves transient formation of a bond at the methyl-/methane carbon position.

If a community comprised of these organisms produced methane *via* an acetate intermediate, and if the CO_2 utilized had an initial isotopic composition of -25‰ *vs.* PDB, use of a small fraction of that pool to produce acetic acid would yield material at about -60‰. Use of a small fraction of that product by acetoclastic organisms would yield CH_4 at -90‰, and use of 50% of that pool would yield CH_4 at -75‰. These results are being prepared for publication, and have already been presented at a research conference sponsored by the American Association of Petroleum Geologists.

Carbon isotope effects in anaerobic photosynthetic bacteria. It is often suggested [A-8, A-9, and references cited therein] that photosynthetic bacteria that utilize electron donors other than H_2O were the first photoautotrophs and served as primary producers of organic matter in the Early Archean ecosystem. In that case, the isotopic contrast observed between Early Archean kerogen and coexisting carbonates ought to record the carbon kinetic isotope effect characteristic of (i) carbon assimilation and (ii) biosynthetic reactions leading from primary photosynthate to the organic residues most resistant to further degradation. The fractionation observed in the geologic record is ~34‰ [A-9], considerably larger than that observed in any investigation of assimilatory fractionation of carbon isotopes by a photoautotroph operating at modern pCO_2.

Since this "fractionation gap" was recognized, various authors [*e. g.*,

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A-9] have sought to bridge it by postulating that Archean photoautotrophs displayed a larger overall isotopic fractionation because they operated at much higher partial pressures of carbon dioxide. Increased fractionation at higher pCO_2 was observed by Park and Epstein (1960) and by Calder and Parker (1973) in studies of aerobic photoautotrophs, and can be inferred from the best presently-available models of C_3 carbon fixation (Farquhar et al., 1982). The situation with anaerobic photoautotrophs is less clear, but not unpromising. Wong et al. (1975) observed fractionations (CO_2 vs. biomass) as large as 29‰ in their study of Chromatium vinosum grown at 40 torr CO_2 (+ N_2 to make a total pressure of 1 atm). This corresponds to a carbonate vs. biomass contrast approaching 36‰, depending upon temperature and pH. It could, therefore, be asserted that, for anaerobic photoautotrophs, there is no fractionation gap. But more extensive evidence would be welcome.

In connection with other investigations (described in later sections), Ray Takigiku, a graduate student supported by this grant, took the opportunity to explore assimilatory fractionation of carbon isotopes by Rhodopseudomonas capsulata, a photosynthetic bacterium that can be grown autotrophically and which happens to be unusually tolerant of high pressures of CO_2. He found that overall fractionation of carbon isotopes varied by a factor of at least four and was strongly dependent upon pressure in the range $0.2 \leq pCO_2 \leq 490$ torr. A maximum fractionation of 23.4‰ was observed at $pCO_2 = 37$ torr (corresponds to 5% of present atmospheric pressure or 150X the present atmospheric level of CO_2). Note that the large fractionation measured by Wong et al. (1975) was observed at $pCO_2 = 40$ torr. Both in our work, and in that of Wong et al., lipids were depleted in ^{13}C relative to biomass, the additional fractionation amounting to 3.9‰ in our case.

We do not suggest that isotopic fractionations imposed by anaerobic photoautotrophs are, or could furnish, some kind of pCO_2 paleobarometer, but the existence of a maximum in the fractionation *vs.* pCO_2 relationship is interesting and bears further investigation.

Nitrogen isotope effect associated with fixation of N_2. The effect is "known" to be essentially zero [A-8]. We became concerned, however, that the effect measured in most, if not all, previous experiments might not reflect the isotope effect associated with fixation but, instead, might record that of mass transport, the overall system being:

$$N_2 \text{ (atm)} \xrightarrow[\text{diffusion}]{} N_2 \text{ (intracellular)} \xrightarrow[\text{nitrogenase}]{} N_{fixed}$$

If mass transport were rate limiting -- and, given the requirement that nitrogenase be protected from atmospheric O_2 by construction of diffusional barriers, as in heterocysts, it seemed that it might be -- any isotope effect associated with nitrogen fixation itself, no matter how large, would be "invisible." The possibility that a significant isotope effect might, in this way, have been overlooked was of interest for two reasons:

(i) The first nitrogen fixers would have developed while the atmosphere and hydrosphere were anoxic. A diffusional barrier for protection of nitrogenase would not have been required. Any isotope effect associated with the action of nitrogenase would, in that case, affect the isotopic composition of fixed nitrogen, and nitrogen-isotopic compositions in Archean kerogens might record that fractionation.

(ii) Working with anaerobic photosynthetic bacteria in Prof. Gest's laboratory, it would be easy for us to measure the isotope effect associated with nitrogenase unprotected by a heterocyst or any other diffusional

barrier.

This measurement was undertaken by Ray Takigiku. For Rhodopseudomonas capsulata at 32°C, he found α(gas/cell) = 1.00223 ± 0.00004. This result is very close to most previous reports, and we conclude that these systems are adequately represented by

$$N_2 \text{ (atm)} \rightleftharpoons N_2 \text{ (intracellular)} \rightarrow N_{fixed}$$

and that the nitrogen kinetic isotope effect associated with the action of nitrogenase is about 0.22‰, so small that the rate-determining step in the reaction mechanism must not involve a significant change in bonding at N.

Nitrogen isotope effects in biosynthesis of tetrapyrroles. For reasons outlined in the research-plans section of this proposal, an ability to recognize certain nitrogen-isotopic compositions in porphyrins as indicative of unique origins would be of considerable value. As a first step in exploring the possibility of that development, Ray Takigiku has measured intracellular nitrogen-isotopic fractionations between tetrapyrroles and other N-containing compounds in Rps. capsulata, C. vinosum, and higher-plant chloroplasts. The chlorophyllides were, in general, slightly enriched (1‰) in ^{15}N.

In the case of M. thermoautotrophicum, Takigiku found that a normal isotope effect of 10.9‰ was associated with assimilation of N (from NH_4Cl in the medium). The tetrapyrrole, F_{430}, was, however, enriched in ^{15}N relative to cellular average N. The difference was large enough that tetrapyrrole N was essentially equal in isotopic composition to substrate (NH_4Cl) N.

The complete biosynthetic pathway for F_{430} is not known. It is conceivable that N assimilated for tetrapyrrole biosynthesis enters the cell as NH_4^+, while remaining N enters as NH_3, and that the fractionation observed is that related to the $NH_4^+ \rightleftharpoons NH_3$ equilibrium isotope effect (which favors accumulation of ^{15}N in NH_4^+, ϵ = 34‰ at 25°C).

Isotope effects in microbial sulfur cycles. The sulfur isotope effect associated with dissimilatory reduction of sulfate by sulfate-reducing bacteria is large and relatively well studied. Most other isotope effects in the sulfur cycle are thought to be small, but are, in fact, poorly known. This situation presents itself with painful clarity to anyone attempting to estimate sulfur-isotopic compositions likely to arise in a hypothetical Archean ecosystem in which sulfur serves as the principal redox partner for carbon. It was with that problem in mind that we gladly accepted Brian Fry's application for a post-doctoral appointment in our laboratories.

During the twenty months that he received half-time support from these funds, Fry measured the sulfur isotope effect associated with anaerobic, photosynthetic oxidation of sulfide by Chromatium vinosum [A-17], the isotope effects associated with oxidation of sulfite and thiosulfate by the same organism [B-4], and sulfur isotope effects associated with the equilibria noted below [C-2].

$$H_2S\ (aq) \rightleftharpoons H_2S\ (g)$$

$$H_2S \rightleftharpoons H^+ + HS^-$$

Knowledge of both of these equilibrium isotope effects is required for interpretation of sulfur isotopic fractionations observed in many natural

systems. Fry also carried out a very interesting investigation of the sulfur-isotopic compositions of deep-sea hydrothermal vent animals [A-13]. The results of this study showed clearly that chemosynthetic bacteria deriving energy by utilization of seawater O_2 for oxidation of vent-derived sulfide were essentially the only autotrophs in the vent communities. For further details of Dr. Fry's work, interested readers are referred to the cited appendices.

Structural and Isotopic Studies of Organic Compounds in Ancient Sediments

The New Albany Shale. Ray Takigiku has undertaken a study of the isotopic compositions of fractions of extracts from the New Albany Shale, a local sediment of Devonian age. The New Albany is a black shale containing up to 30% organic carbon. Though clearly of marine origin, the organic material is depleted in ^{13}C relative to that found in modern ocean water. Sedimentological and stratigraphic investigations by Maynard (1981) have shown that the abundance of ^{13}C is directly correlated with terrigenous inputs. This correlation is "backwards" according to much conventional wisdom, which holds that marine organic material always contains more ^{13}C than terrestrial material. The mechanism for depletion of ^{13}C in black shales is unknown, but of broad interest, bearing strongly on the now-famous Cretaceous "anoxic events" and, possibly, on the "excess depletion" of ^{13}C generally observed in Precambrian sediments. The problem has been discussed in some detail by Hailer, Leininger, and Hayes [A-15].

Extracts of the New Albany Shale are brilliantly pigmented by extraordinarily abundant porphyrins. We wish particularly to examine the possibility that these porphyrins are, in part, at least, related to F_{430}, the tetrapyrrole synthesized by methanogens. We suspect that contents of

organic matter in the New Albany sediments were so high that methanogenic fermentation played a significant role in organic diagenesis. Reincorporation of carbon from bacteriogenic methane or accumulation of ^{13}C-depleted methanogen biomass may have contributed to the observed isotopic lightness.

In order to examine these possibilities, Takigiku is working to determine carbon- and nitrogen-isotopic compositions of compounds and fractions of particular interest. His studies are not yet complete and results are, therefore, unpublished and difficult to interpret. We will summarize them briefly here, and discuss plans for further work, including investigations of diagenetic carbonates and sulfides, in the research-plans section of this proposal. It should be noted that this work is related to the previously-described studies of carbon- and nitrogen-isotopic fractionation in methanogens.

Crude extract fractions (bitumens, asphaltenes) are slightly enriched (2‰) in ^{13}C relative to coexisting kerogen at the base of the New Albany section, approximately equal at the top. Compositions average ~ -30‰ vs. PDB. Nitrogen isotopic compositions are equal in all fractions, ~ +2‰ vs. air. H/C for the kerogen is 1.2. Resolution of the bitumens into six subfractions shows that the least polar material (alkanes) is most strongly depleted in ^{13}C, but the differences among all subfractions are small, amounting only to 1.5‰. Division of the alkanes into normal, branched-cyclic, thiourea adduct, and thiourea non-adduct fractions reveals a maximum isotopic contrast of 1.2‰ (n-alkanes are lightest, cyclic polyisoprenoids heaviest). Porphyrins from the relatively carbon-poor lower portion of the section are enriched in ^{13}C relative to coexisting kerogen by

as much as 5‰. In contrast, those from the carbon-rich Henryville Bed are not enriched in ^{13}C relative to the still-lighter kerogen in that part of the section. There are no strongly-evident spectral differences between the porphyrin fractions, in spite of the isotopic contrast.

Pure compounds from miscellaneous sediments. In order to gain further information on intermolecular isotopic contrasts in sedimentary mixtures, we have undertaken a program of measurements of pure organic compounds provided to us by Dr. Pierre Albrecht of the Universite' Louis Pasteur in Strasbourg, France. Analysis of a 4-methyl stanol, recovered from the Messel Shale and thought to be derived either from dinoflagellates or methylotrophic bacteria, revealed an isotopic composition of -28.05‰ vs. PDB, a result quite decisively not in favor of a methylotrophic origin for this material, but near the minimum ^{13}C content expected for material biosynthesized in the water column. Other materials ranged in composition from -35.03 to -25.32‰. This work promises to provide useful baseline information for interpretation of isotopic compositions of pure sedimentary organic compounds, and will be continued as samples become available.

Developments in Isotopic Geochemistry and Analyses

Carbon-isotopic studies. At the urging (!) of Hyman Hartman, our group joined that of Bernd Simoneit in a study of organic material from the Red-Sea Deeps. It had been suggested (e. g., Dowler and Ingmanson, 1979) that organic material found beneath these hot, sterile brines was of abiotic origin. Results of our study, now prepared for publication as a full paper [C-1], show clearly that the material is biotic debris from the overlying normal marine water column. The study has interest as an example of completely abiotic diagenesis, and discussions contributed by Simoneit consider

this point in some detail.

Finding that the literature contains few, if any, discussions of the "nuts and bolts" of isotopic techniques useful in organic geochemistry, Hayes has prepared several textbook-style reviews [A-4, A-16]. Unfortunately, the second, more-extensive and better-documented review wound up appearing in a set of notes for an SEPM short course that was canceled due to lack of enrollment (that happening, no doubt, because the organizers of the short course missed the pre-conference advertising deadline). Recently, the editors of Die Naturwissenschaften have said that they would be glad to consider publishing a slightly "de-textbookized" version of that manuscript.

Brian Fry prepared an extensive review of carbon-isotopic techniques for the investigation of food webs. Several pages are included in the appendix of this report [A-20].

Techniques for analysis of hydrogen isotopes. Special problems arise in the measurement of natural variations of hydrogen-isotopic abundances. Mass spectrometry is complicated by formation in the ion source of H_3^+, an ion with the same mass as the isotopic species of interest, HD. Failure to take contributions by H_3^+ to the mass-3 ion beam adequately into account can lead to serious systematic errors, but analysts are often so impressed by the high precision with which ion-current ratio measurements can be made that they fail to consider systematic errors carefully enough. The technique described by Schoeller, Peterson, and Hayes [A-6] involves simultaneous comparison of a sample with two reference gases of known isotopic composition and makes possible calculations that are immune to errors asso-

ciated with inconstant or incorrectly measured H_3^+ contributions.

Isotope ratios of carbon and nitrogen can be determined directly from the products of combustion of organic material. In the case of hydrogen, however, the water which forms as a product of combustion is not suitable as a mass spectrometric sample gas. Reduction of the water to H_2 has conventionally been carried out by reaction with uranium metal at 750°C. That technique has some points of danger, but many more of inconvenience. Leaks of air into the vacuum system result in inactivation of the uranium by formation of uranium oxide and nitride. Hydrogen gas is highly soluble in uranium metal, and long periods of Toepler pumping are required for collection of the reduction product. The uranium oxide which forms as a result of reaction with water is extremely mobile within vacuum systems and poses a low-level radiation hazard and can act as sorbent for H_2 and H_2O outside the hot zone of the reactor.

For all of those reasons, there was considerable interest in the announcement by Coleman et al. (1983) that a satisfactory procedure for use of zinc metal as a water reductant had been developed. Unfortunately, it has turned out that Coleman's procedure requires a "special" zinc shot that apparently contains a crucial impurity and/or has a unique surface structure. About two years ago, we learned from colleagues at the U. S. Geological Survey that the Coleman zinc contained lead as an impurity. After encouraging them to publish their finding and to synthesize an ideal, optimally-impure zinc, we waited for more than a year before undertaking work ourselves. Feeling quite sure that a useful and commercially important reagent could be developed in short order, we obtained $4,000 via the PI's consulting relationship with Finnigan MAT to support the summer work of Eric

Wachter, an outstanding undergraduate interested in the problem.

We have learned that our colleagues at the Geological Survey have returned to the use of the "special" zinc, and we think we understand why. Lead is certainly not the secret. Wachter was able to develop a workable procedure based on Pb-Zn mixtures, but not anything that works as well as the Coleman zinc (which is, fortunately, still available as Hopkins and Williams Analyzed Reagent). The availability of a good procedure for reduction of H_2O is, however, crucial to further development of hydrogen-isotopic lines of inquiry in biogeochemistry, and we have continued work on this problem. It appears that we have now come very close to a satisfactory solution to this problem. A report describing our results in included as Appendix G-4. Because the possibility still exists that this may lead to a valuable product (now to the benefit of the university and NASA as well as Finnigan), the report specifically excludes mention of the mystery component.

Oxygen-isotopic investigations. In completing his thesis work, K. W. Wedeking, a student supported by this grant, developed evidence that appears to support strongly the idea that the origin of oxygenic photosynthesis -- or some episode of oxygenation, at least -- occurred 2.8 Ga ago. In brief, we found that the $^{18}O/^{16}O$ ratio in organic matter varies dramatically at that point in time, and developed an interpretation linking that observation to the first appearance of free O_2 in the hydrosphere. Wedeking's work on this subject comprised three phases: (i) development of a technique for the analysis of the isotopic composition of oxygen in organic matter, (ii) exploration of oxygen-isotope exchange phenomena in kerogens and related

materials, and (iii) oxygen-isotopic analysis of a suite of Precambrian and Phanerozoic kerogens. Unfortunately, much of this work remains unpublished. A summary report follows.

Although several recent publications (cited in the attached manuscript, appendix G-5) have reported methods for the analysis of oxygen isotopes in cellulose, a material of special interest in paleoclimatic investigations, development of a technique specifically applicable to kerogens was required. Cellulose contains much oxygen, can be easily purified, and is usually available in abundance (even if material from a single tree ring is to be analyzed). Kerogen, in contrast, contains little oxygen, always contains some nitrogen and sulfur in addition, is usually contaminated by pyrite and trace minerals, and is frequently available only in very small quantities. Analytical methods developed for application to cellulose had to be made to function at a far-lower sample quantity (lower blanks and higher yields were required) and in the presence of a variety of possible interferents. We thought we had accomplished these goals, and prepared a report for publication [G-5].

A reviewer of that manuscript, however, pointed out that nitrogen interferences might have affected some test analyses of proteins (which are, of course, very rich in N), and we decided to modify the procedure to avoid that possibility. Wedeking's apparatus was dismantled in the course of the modifications and, due to lack of student interest in the problem, has never been reassembled. As a result, the analytical method remains undocumented.

The phenomenon of oxygen-isotopic exchange can be addressed separately. Exchange between water and functional groups in organic compounds has been

studied in some detail (see references cited in reprint A-14), but never with materials or under conditions clearly relevant to these investigations. Therefore, we studied (i) exchange processes possibly occurring during the isolation and purification of the kerogen (*i. e.*, throughout the course of sample handling), and (ii) exchange processes possibly occurring during storage and "maturation" of the kerogen in the sediment. In the first of these processes, enormous excesses of oxygen (in the form of water) are present together with high concentrations of strong mineral acids. Conditions may be less severe in sediments (the second process), but times are, literally, geological, and rather high temperatures may be reached.

Wedeking utilized materials enriched in ^{18}O in order to study exchange phenomena. We see no way in which any deficiencies in the analytical procedure could significantly have affected these results, and have proceeded with their publication [A-14]. In brief, they show that the kinetics of oxygen isotopic exchange processes investigated forty years ago by Urey were, in fact, correctly measured (though that should hardly be a surprise) and that, using more sensitive, modern techniques, the presence of an equilibrium isotope effect can also be demonstrated. The techniques tested in that way were further applied to a study of the exchange of oxygen isotopes between water and crude biological material, and it was shown that approximately two-thirds of the oxygen remaining in the insoluble organic material at any time had not been exchanged and was isotopically representative of the oxygen in the starting organic material. It is, thus, expected that a significant portion of the oxygen in sedimentary organic matter will have an isotopic composition representative of the initially deposited material.

The isotopic composition of oxygen in a wide variety of kerogens and humic acids (we acknowledge with thanks the provision of Phanerozoic materials by D. H. Stuermer and I. R. Kaplan, UCLA; and by B. Durand, Institut Francais du Petrole) was investigated in the last phase of Wedeking's work. We found that, apart from a minor and not-unexpected dependence of isotopic composition on the $^{18}O/^{16}O$ ratio in local water, the oxygen-isotopic composition of sedimentary organic matter has been quite consistent over geologic time. The only noticeable fluctuation observed in more than 100 analyses was found in kerogens with an age of 2.8 Ga.

Even before anything was known of the oxygen-isotopic variations, the carbon-isotopic record had been interpreted [A-9] in terms of the development of oxygen-producing photosynthesis 2.8 Ga ago. This was, of course, very much in our mind as we considered the oxygen-isotopic data. The observed "isotopic signal" requires that some non-exchangeable oxygen pool (i. e., one that could not be equilibrated with oxygen isotopes in water) with an exotic isotopic composition must have been present 2.8 Ga ago. Molecular O_2 is one of the few species that might fulfill this requirement, and it can be suggested that its isotopic composition was "exotic" because the great majority was being promptly consumed by a process with a significant isotope effect, namely the oxidation of ferrous iron, which appears to have been abundant in the ocean at that time. Later, the "crustal sink" for O_2 was less potent, O_2 began to accumulate, and isotopic compositions began more closely to resemble modern values.

We did not want to publish findings that were bound to be considered remarkable without (i) being able to point to documentation of the analytical procedure and, preferably, (ii) confirming the result. Up to now, this

[illegible]. Attempts to utilize the alternative analytical procedure [illegible] by Sentruck (see introduction, EPA-related work) have failed because the nitrogens are too poor in O (and, perhaps, too refractory). We have no reason to believe the initial results are inaccurate either because of nitrogen interferences (which are very unlikely to have been a problem with these very N-poor materials) or for any other reason. But the question is of such magnitude -- and maintenance of a standard of accuracy is so important within the field of isotope geochemistry -- that we will not publish these results until they have been confirmed. Plans for further work are outlined in a later section of this proposal.

Treatment of carbonates with phosphoric acid in order to produce CO_2 for isotopic analysis of oxygen and carbon has been been a mainstay of isotope geochemistry for 35 years. As analytical chemists, however, we were troubled by some obvious problems. "100% H_3PO_4," the reagent utilized in the conventional procedure, is, in fact, a mixture of H_3PO_4, various anhydride polymers, and water. Why, then, are there supposedly "no problems" with exchange of oxygen isotopes between CO_2 and those traces of H_2O? Could it be that some exchange does, in fact, occur, and that this is a significant cause of imprecision in the analytical methods? Might this exchange be demonstrated and, with detailed information in hand, a better procedure developed -- even for something as hoary as the phosphorolysis of carbonates? Would I ask all these questions if the answers weren't yes? Refer to [B-1], and please excuse the cute remark.

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